Endiandric Acid Analogues from the Roots of *Beilschmiedia erythrophloia*

Ping-Shin Yang,[†,#] Ming-Jen Cheng,[‡,#] Chien-Fang Peng,[§] Jih-Jung Chen,[⊥] and Ih-Sheng Chen*[,†]

Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan 807, Republic of China, Bioresource Collection and Research Center (BCRC), Food Industry Research and Development Institute, Hsinchu, Taiwan 300, Republic of China, Department of Medical Laboratory Science and Biotechnology, College of Health Sciences, Kaohsiung Medical University, Kaohsiung, Taiwan 807, Republic of China, and Graduate Institute of Pharmaceutical Technology and Department of Pharmacy, Tajen University, Pingtung, Taiwan 907, Republic of China

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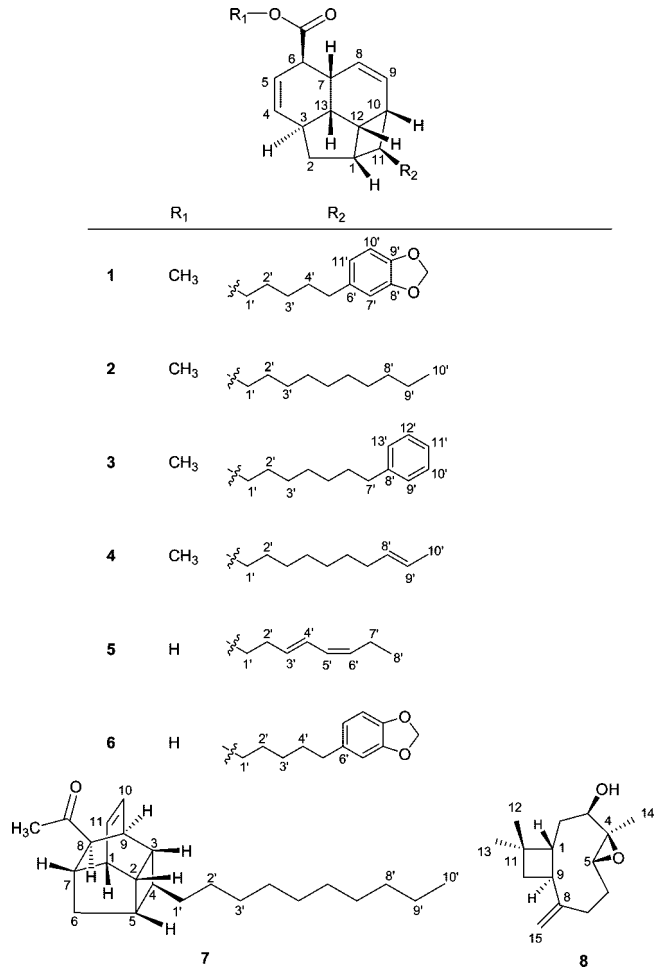
Investigation of the roots of *Beilschmiedia erythrophloia* has led to the isolation of seven new endiandric acid analogues, erythrophloins A−F (**1**−**6**) and beilcyclone A (**7**), together with 11 known compounds. The structures of **1**−**7** were determined using spectroscopic techniques. Two constituents, erythrophloin C (**3**) and suberosol B (**8**), exhibited antitubercular activity against *Mycobacterium tuberculosis* H37Rv, showing MIC values of 50 and 28.9 μg/mL, respectively.

Beilschmiedia erythrophloia Hay. (Lauraceae) is an evergreen tree, distributed throughout south mainland China, Hainan Island, the Ryukyus, and Taiwan.[1] In previous studies, endiandric acids A−D were found to occur in two species of *Endiandra*[2−6] (Lauraceae) and three species of *Beilschmiedia* from Australia.[6] There are, however, only two species of *Beilschmiedia* in Taiwan. Several cytotoxic and antitubercular constituents have been isolated, from the stem[7] and the leaves,[8] respectively, from Formosan *B. tsangii*, but no endiandric acid analogues were found to be present. Recently, over 1000 Formosan plants have been screened for in vitro antitubercular activities, and *B. erythrophloia* was found to be an active species, but its chemical constituents have never been studied. Investigation of the active EtOAc-soluble fraction of the root of *B. erythrophloia* has led to the isolation of seven new endiandric acid analogues, erythrophloins A−F (**1**−**6**) and beilcyclone A (**7**), along with 11 known compounds, including one sesquiterpene, suberosol B (**8**),[9] an amide dioxamine,[10] the benzenoid vanillin,[11] the steroids 6β-hydroxystigmast-4-en-3-one[12] and 24(*S*)-3β-hydroxystigmast-5-en-7-one,[13] the triterpenes lupeol[14] and 3-*O*-acetyl-*epi*-betulinic acid,[15] the benzoquinone α-tocopheryl quinone,[16] and three fatty acid esters, methyl oleate,[17] methyl palmitate,[18] and methyl linoleate.[19] The structural elucidation of compounds **1**−**7** was based on spectroscopic data analysis, and the known compounds were identified by comparison with data from the literature. We report herein on the isolation and structural elucidation of **1**−**7** and on the antitubercular activities of the isolates from *B. erythrophloia*.



Results and Discussion

Compound **1** was isolated as an optically inactive yellowish oil. The HRESIMS gave a $[M + Na]^+$ ion peak at *m/z* 443.2196 (calcd 443.2198), consistent with a molecular formula of $C_{27}H_{32}O_4$. The ^{13}C NMR (Table 1) and DEPT spectra exhibited 27 signals for one methyl, seven methylenes, 15 methines, and four quaternary carbons. UV absorptions of a benzenoid nucleus at 239 and 287 nm and the methylenedioxy bands at 1039 and 934 cm^{-1} in the IR spectrum, together with the ^1H NMR signals (Table 2) for one methylenedioxy at δ 5.91 (s) and an ABX system at δ 6.61 (1H, dd, J = 8.0, 1.6 Hz, H-11′), 6.67 (1H, d, J = 1.6 Hz, H-7′), and 6.72 (1H, d, J = 8.0 Hz, H-10′), suggested the presence of a methylenedioxyphenyl moiety. In addition to this moiety observed from the ^{13}C NMR and DEPT spectra, there were 12 tertiary C atoms, including four olefinic carbons in the remaining structure of **1**. These *cis*-form olefinic protons were evident at δ 5.45 (1H, ddd, J = 10.0, 3.2, 1.6 Hz, H-8), 5.62 (1H, ddd, J = 10.0, 4.0, 2.8 Hz, H-9), 5.70 (1H, dt, J = 9.6, 3.0 Hz, H-5), and 6.19 (1H, dt, J = 9.6, 2.4 Hz, H-4). Three fragments of contiguous protons from **A** (H-9, H-8, H-7 [δ 2.95 (m)], H-13 [δ 1.72 (ddd, J = 10.4, 7.2, 5.2 Hz)], H-12 [δ 2.64 (q, J = 8.0 Hz)], H-1 [δ 2.22 (m)], H-11 [δ

* To whom correspondence should be addressed. Tel: (+886)-(0)7-312-1101, ext. 2191. Fax: (+886)-(0)7-321-0683. E-mail: m635013@kmu.edu.tw.
[#] Co-first authors who contributed equally to this work.
[†] College of Pharmacy, Kaohsiung Medical University.
[‡] Food Industry Research and Development Institute.
[§] College of Health Sciences, Kaohsiung Medical University.
[⊥] Tajen University.

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Table 1. ^{13}C NMR Data for Compounds **1**−**6** (in $CDCl_3$, 100 MHz)[a]

position	1	2	3	4	5	6
1	34.9 (CH)	35.0 (CH)	35.0 (CH)	34.9 (CH)	34.8 (CH)	34.9 (CH)
2	34.8 (CH_2)	34.8 (CH_2)	34.8 (CH_2)	34.8 (CH_2)	34.6 (CH_2)	34.7 (CH_2)
3	36.9 (CH)	36.9 (CH)	36.9 (CH)	36.9 (CH)	36.9 (CH)	36.9 (CH)
4	134.1 (CH)	134.1 (CH)	134.2 (CH)	134.6 (CH)	134.5 (CH)	134.5 (CH)
5	124.4 (CH)	124.4 (CH)	124.4 (CH)	123.8 (CH)	123.9 (CH)	123.8 (CH)
6	49.4 (CH)	49.6 (CH)	49.5 (CH)	49.2 (CH)	49.0 (CH)	49.2 (CH)
7	32.8 (CH)	32.9 (CH)	32.9 (CH)	32.8 (CH)	32.8 (CH)	32.8 (CH)
8	129.8 (CH)	129.8 (CH)	129.8 (CH)	129.5 (CH)	129.7 (CH)	129.6 (CH)
9	129.3 (CH)	129.4 (CH)	129.4 (CH)	129.5 (CH)	129.3 (CH)	129.4 (CH)
10	41.2 (CH)	41.2 (CH)	41.2 (CH)	41.2 (CH)	41.1 (CH)	41.1 (CH)
11	45.9 (CH)	46.0 (CH)	46.0 (CH)	46.0 (CH)	45.4 (CH)	45.9 (CH)
12	33.0 (CH)	33.1 (CH)	33.1 (CH)	33.1 (CH)	33.0 (CH)	33.0 (CH)
13	42.0 (CH)	42.1 (CH)	42.1 (CH)	42.0 (CH)	42.0 (CH)	42.0 (CH)
1′	37.0 (CH_2)	37.2 (CH_2)	37.2 (CH_2)	37.2 (CH_2)	36.9 (CH_2)	37.0 (CH_2)
2′	26.8 (CH_2)	27.0 (CH_2)	27.0 (CH_2)	27.0 (CH_2)	30.1 (CH_2)	26.8 (CH_2)
3′	29.0 (CH_2)	29.1−29.7 (CH_2)	29.3 (CH_2)	29.1−29.7 (CH_2)	131.8 (CH)	29.0 (CH_2)
4′	31.7 (CH_2)	29.1−29.7 (CH_2)	29.5 (CH_2)	29.1−29.7 (CH_2)	130.6 (CH)	31.7 (CH_2)
5′	35.6 (CH_2)	29.1−29.7 (CH_2)	29.5 (CH_2)	29.1−29.7 (CH_2)	129.2 (CH)	35.6 (CH_2)
6′	136.6 (C)	29.1−29.7 (CH_2)	31.5 (CH_2)	29.1−29.7 (CH_2)	134.1 (CH)	136.7 (C)
7′	108.8 (CH)	29.1−29.7 (CH_2)	36.0 (CH_2)	32.6 (CH_2)	25.6 (CH_2)	108.8 (CH)
8′	147.4 (C)	32.6 (CH_2)	142.9 (C)	131.6 (CH)	13.6 (CH_3)	147.4 (C)
9′	145.3 (C)	22.7 (CH_2)	128.2 (CH)	124.5 (CH)		145.3 (C)
10′	108.0 (CH)	14.1 (CH_3)	128.4 (CH)	17.9 (CH_3)		108.0 (CH)
11′	121.0 (CH)		125.6 (CH)			121.0 (CH)
12′			128.4 (CH)			
13′			128.2 (CH)			
OCH_2O	100.6 (CH_2)					100.6 (CH_2)
OCH_3	52.0 (CH_3)	52.1 (CH_3)	52.1 (CH_3)			
C=O	175.0 (C)	175.1 (C)	175.0 (C)	180.0 (C)	179.2 (C)	180.6 (C)

[a] All assignments were confirmed by the DEPT, ^{1}H−^{1}H COSY, and HSQC spectra.

1.42 (m)], H-10 [δ 2.25 (m)]), **B** (H-6 [δ 2.99 (m)], H-5, H-4), and **C** (H-13, H-3 [δ 2.53 (m)], H-2 [δ 1.30 (m)]) were revealed from the ^{1}H−^{1}H COSY (Figure 1) and HSQC spectra. They were further connected through the ^3J correlations of the HMBC spectrum (Figure 1). Correlations of H-9 to C-12, H-5 to C-7, H-6 to C-13, H-4 to C-13, H-2α to C-4 and C-11, H-2β to C-4 and C-11, and H-3 to C-12 were used to connect the three fragments, **A**−**C**, and helped establish a tetracyclic structural unit composed of a four-membered, a five-membered, and two six-membered rings for **1**. Also, signals for 12 methines (H-1, H-3−H-13) and one methylene (H-2) indicated that compound **1** is an endiandric acid analogue.[2−4] An ester carbonyl group in the molecule was shown by the band at 1734 cm^{-1} in the IR spectrum and by the signal at δ 175.0 in the ^{13}C NMR spectrum. The presence of a COOMe group was revealed by HMBC correlations between the Me group (δ 3.71) and the C=O group (δ 175.0), and the ^2J and ^3J correlations between a C=O group and both H-6 and H-7 established the connectivity of the methyl carboxylate group at C-6. Finally, the HMBC correlations of H-1′/C-1, H-4′/C-6′, H-11′/C-5′, and H-10′/C-6′ indicated that the main structure and a methylenedioxyphenyl moiety were connected by the remaining five methylenes [δ 1.22 (2H, m, H-2′), 1.26 (2H, m, H-3′), 1.54 (2H, m, H-4′), and 2.51 (2H, t, J = 7.6 Hz, CH_2-5′)] at C-11 and C-6′, respectively. The above assignments were verified by NOESY correlations between H-5′/H-7′ and H-11′ and between H-1′/H-1 and 10. Full assignments of the carbon resonances based on HSQC and HMBC techniques are presented in Table 1. There were eight chiral carbons in the asymmetric structure of **1**. However, in view of its optical rotation, **1** exhibited optical inactivity; thus **1** should be racemic, the same as the reference compound endiandric acid A.[2,3]

Comparison with endiandric acid A[2,3] and the NOESY correlations (Figure 2) confirmed the relative configuration of **1**. The torsional angle (θ) between H-5 and H-6 in endiandric acid A is 81.3°, as obtained from X-ray crystallographic data, with a J value of 2.1 Hz calculated by the Pachler and Wessels equation ($J = 12 \cos^2 \theta - \cos \theta + 2$).[2] The J value of H-5 and H-6 in **1** was 3.0 Hz; thus their torsional angles θ should be smaller than 81.3°, according to the Pachler and Wessels equation. We estimated that H-6 must have an α orientation from the torsional angle (θ) between H-5 and H-6, and H-7 would have a β-orientation based on the absence of any NOESY effect with H-6. The other key NOESY correlations between H-7/H-13, H-13/H-12, H-12/H-1, H-10, and H-1/H-10 were used to establish that H-13, H-12, H-10, and H-1 all adopt a *cis*-β-orientation in **1**. Furthermore, no detectable NOESY effect could be observed between H-3/H-13 and H-11/H-10, H-1, thereby supporting the α-orientation of the protons at H-3 and H-11. In summary, the relative configuration of **1** was proposed as *rel*-(1*S*,3*S*,6*R*,7*R*,10*R*,11*S*,12*S*,13*S*),[2,3] and this compound was named erythrophloin A.

Compounds **2** and **3** were both obtained as yellowish oils. The molecular formulas of **2** ($C_{25}H_{38}O_2$) and **3** ($C_{28}H_{36}O_2$) were established by the $[M]^+$ ion peak at m/z 370.2861 and the $[M + Na]^+$ ion peak at m/z 427.2610 in the HREIMS and in the HRESIMS, respectively. The ^{13}C/^{1}H NMR (Tables 1 and 2), COSY (Figure 1), HSQC, and HMBC spectra (Figure 1) of **2** and **3** were similar to **1** and also contained 13 skeletal signals of an endiandric acid moiety, including the presence of 12 methines and one methylene. The characteristic four *cis* olefinic protons at δ 5.44 (1H, ddd, J = 10.0, 3.3, 1.6 Hz, H-8), 5.64 (1H, ddd, J = 10.0, 4.2, 2.7 Hz, H-9), 5.70 (1H, dt, J = 9.6, 3.0 Hz, H-5), and 6.19 (1H, dt, J = 9.6, 2.4 Hz, H-4) in **2** were almost the same as in **1**, but the signals for a methylenedioxyphenyl moiety in **1** were absent in **2**. In the ^{1}H NMR spectrum of **2**, the signals at δ 1.22 (2H, m, H-2′), 1.26 (14H, br s, H-3′-H-9′), and 1.48 (2H, m, H-1′) and a terminal methyl [δ 0.88 (3H, t, J = 6.9 Hz, H-10′)] revealed that a decyl group is located at C-11, according to the ^3J correlation of H-1′/C-1′ in the HMBC spectrum (Figure 1). In addition, a terminal methyl signal in **2** could not be observed in **3**. In addition to the endiandric acid moiety, the ^{13}C NMR (Table 1) and DEPT spectra of **3** also exhibited five aromatic protons and one quaternary carbon [δ 142.9 (C-8′)], and the ^{1}H NMR spectrum showed signals of these five aromatic protons at δ 7.17 (3H, m, H-10′, 11′, 12′) and 7.27 (2H, m, H-9′, 13′). Accordingly, a monosubstituted phenyl group in **3** replaced the methylenedioxyphenyl group in **1**. According to the ^3J correlations between H-1′/C-1 and H-7′/C-9′ and C-13′ in the HMBC spectrum and the NOESY correlations between H-1′/

Table 2. ^{1}H NMR Data for Compounds **1**−**6** (in $CDCl_3$, 400 MHz)

	δ_H (J in Hz)					
position	**1**	**2**	**3**	**4**	**5**	**6**
1	2.22, m	2.24, m	2.22, m	2.26, m	2.25 m	2.23, m
2α	1.30, m	1.33, m	1.33, m	1.32, m	1.33, td (12.8, 5.6)	1.33, m
2β	1.56, m	1.57, m	1.59, m	1.58, dd (11.6, 5.6)	1.59, dt (6.4, 5.2)	1.56, m
3	2.53, m	2.56, m	2.53, m	2.56, m	2.56, m	2.57, m
4	6.19, dt (9.6, 2.4)	6.19, dt (9.6, 2.4)	6.19, dt (9.6, 2.4)	6.22, dt (9.6, 2.6)	6.22, dt (9.6, 2.4)	6.22, dt (10.0, 2.6)
5	5.70, dt (9.6, 3.0)	5.70, dt (9.6, 3.0)	5.70, dt (9.6, 3.2)	5.73, dt (9.6, 2.6)	5.74, dt (9.6, 3.0)	5.73, dt (10.0, 2.6)
6	2.99, m	2.99, m	3.00, m	3.03, m	3.04, m	3.02, m
7	2.95, m	2.95, m	2.95, m	3.00, m	3.00, m	2.99, m
8	5.45, ddd (10.0, 3.2, 1.6)	5.44, ddd (10.0, 3.3, 1.6)	5.45, ddd (10.4, 3.2, 1.6)	5.46, ddd (10.0, 3.2, 2.0)	5.46, ddd (10.0, 3.2, 1.6)	5.45, ddd (10.0, 3.2, 1.6)
9	5.62, ddd (10.0, 4.0, 2.8)	5.64, ddd (10.0, 4.2, 2.7)	5.64, ddd (10.4, 4.0, 3.2)	5.65, ddd (10.0, 3.6, 3.2)	5.65, ddd (10.0, 4.0, 2.8)	5.63, ddd (10.0, 4.0, 2.8)
10	2.25, m	2.27, m	2.25, m	2.26, m	2.29, m	2.27, m
11	1.42, m	1.46, m	1.45, m	1.46, m	1.48, m	1.42, m
12	2.64, q (8.0)	2.64, q (7.8)	2.64, q (8.0)	2.64, q (8.0)	2.66, q (7.2)	2.64, q (7.2)
13	1.72, ddd (10.4, 7.2, 5.2)	1.72, ddd (12.0, 7.8, 5.7)	1.72, ddd (10.4, 7.2, 5.2)	1.72, br ddd (11.2, 7.6, 4.8)	1.73, br ddd (10.4, 7.2, 5.2)	1.72, ddd (10.0, 7.2, 5.2)
1′	1.44, m	1.48, m	1.45, m	1.46, m	1.59, dt (6.4, 5.2)	1.46, m
2′	1.22, m	1.22, m	1.30, m	1.26, s	2.01, q (7.2)	1.23, m
3′	1.26, m	1.26, br s	1.30, m	1.26, s	5.54, dt (14.4, 7.2)	1.28, m
4′	1.54, m	1.26, br s	1.30, m	1.26, s	5.99, m	1.54, m
5′	2.51, t (7.6)	1.26, br s	1.30, m	1.26, s	5.99, m	2.51, t (7.2)
6′		1.26, br s	1.30, m	1.26, s	5.61, dt (10.0, 7.2)	
7′	6.67, d (1.6)	1.26, br s	2.60, t (7.6)	1.96, m	2.08, quin (7.2)	6.67, d (1.6)
8′		1.26, br s		5.41, m	1.00, t (7.2)	
9′		1.26, br s	7.27, m	5.41, m		
10′	6.72, d (8.0)	0.88, t (6.9)	7.17, m	1.64, d (10.0)		6.72, d (8.0)
11′	6.61, dd (8.0, 1.6)		7.17, m			6.61, dd (8.0, 1.6)
12′			7.17, m			
13′			7.27, m			
OCH_2O	5.91, s					5.91, s
OCH_3	3.71, s	3.71, s	3.72, s			

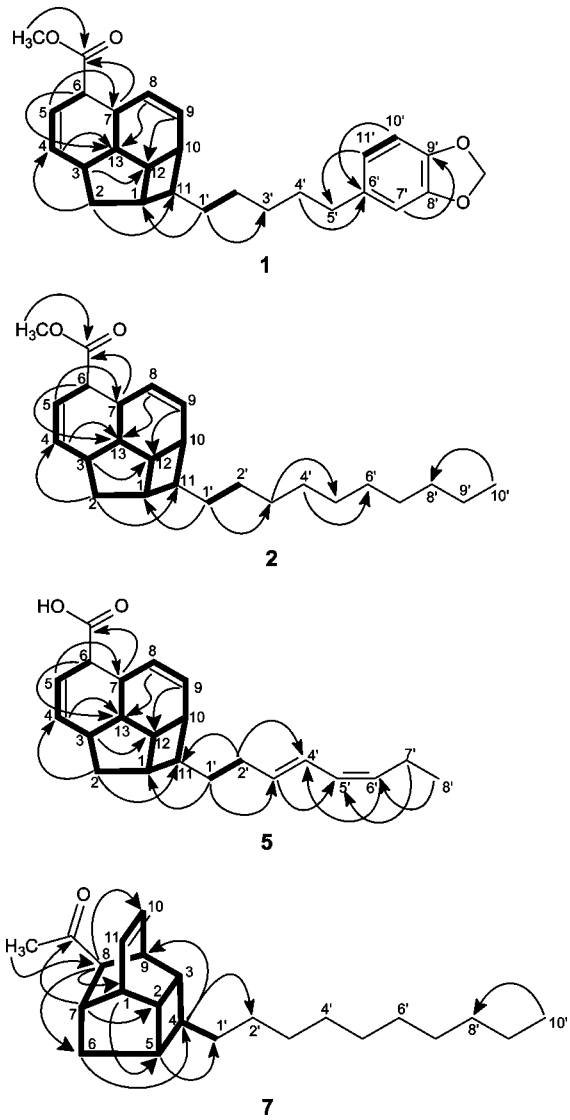

Figure 1. Key ^{1}H−^{1}H COSY (—) and HMBC (H→C) correlations of **1**, **2**, **5**, and **7**.

H-1 and H-10, H-6′/H-13′, and H-7′/H-9′ it could be seen that the seven methylenes [δ 1.22−1.37 (10H, m, H-2′-H-6′), 1.45 (2H, m, H-1′), and 2.60 (2H, t, J = 7.6 Hz, H-7′)] were connected to the phenyl moiety and the main structure at C-8′ and C-11 in **3**, respectively. Because of their optical inactivity, **2** and **3** were considered, like **1**, to be racemic. The relative configurations of **2** and **3** were determined by NOESY correlations and comparison with endiandric acid A.[2,3] Their relative configurations were the same as **1**. Thus, the structures of **2** and **3** were established as shown and were named erythrophloins B and C, respectively.

Compounds **4**−**6** were all isolated as optically inactive yellowish oils. The HRESIMS of **4** afforded a [M + Na]$^+$ ion peak at *m/z* 377.2453, suggesting a molecular formula of $C_{24}H_{34}O_2$. Similar to **1**−**3**, their ^{13}C/^{1}H NMR spectra (Tables 1 and 2) also revealed the characteristic four *cis* olefinic protons and also showed that compounds **4**−**6** contain 13 skeletal signals of a tetracyclic endiandric acid moiety. A carbonyl group was indicated by the band at 1721 cm^{-1} in the IR spectrum of **4** and confirmed by the signal at δ 180.0 in the ^{13}C NMR spectrum. Besides characteristic endiandric acid olefinic proton signals appearing at δ 5.46 (1H, ddd, J = 10.0, 3.2, 2.0 Hz, H-8), 5.65 (1H, ddd, J = 10.0, 3.6, 3.2 Hz, H-9), 5.73 (1H, dt, J = 9.6, 2.6 Hz, H-5), and 6.22 (1H, dt, J = 9.6, 2.6 Hz, H-4), two olefinic protons also appeared at δ 5.41 (2H, m, H-8′, H-9′), together with a vinyl methyl observed at δ 1.64 (3H, d, J = 10.0 Hz, H-10′) and seven methylenes, representing a dec-2-enyl side chain moiety located at C-11. This was confirmed by observation of the ^3J correlation between H-1′ and C-1 in the HMBC spectrum. The double bond on C-8′, 9′ was assigned as *trans* by the chemical shift of C-7′ at δ 32.6 in the ^{13}C NMR spectrum in **4**.[20] Together with the endiandric acid olefinic proton signals, the ^{1}H NMR spectrum of **5** showed another four olefinic protons [δ 5.54 (1H, dt, J = 14.4, 7.2 Hz, H-3′), 5.99 (2H, m, H-4′, H-5′), and 5.61 (1H, dt, J = 10.0, 7.2 Hz, H-6′)], from the side chain moiety of **5**, with the remaining signals being three methylenes [δ 1.59 (2H, dt, J = 6.4, 5.2 Hz, H-1′), 2.01 (2H, q, J = 7.2 Hz, H-2′), 2.08 (2H, quin., J = 7.2 Hz, H-7′)] and a terminal methyl [δ 1.00 (3H, t, J = 7.2 Hz, H-8′)]. HMBC correlations of H-1′/C-3′, H-2′/C-4′, H-3′/C-5′, H-6′/C-4′, H-7′/C-5′, and H-8′/C-6′ revealed the side chain to be composed of an octa-3′,5′-dienyl moiety, attached to the main skeleton at C-11, through the HMBC correlations of H-1/C-1′ and H-2′/C-11. The 3′*E*,5′*Z* geometry was ascertained according to the ^{13}C NMR signals of δ 30.1 (C-2′) and 25.6 (C-7′) together with the coupling constants (J = 14.4 Hz) of H-3′ and H-4′ (δ 5.54 and 5.99) and the coupling constants (J = 10 Hz) of H-5′ and H-6′ (δ 5.99 and 5.61). The hydroxyl and carbonyl group absorptions at 2600−3300 and 1701 cm^{-1} in the IR spectrum and the ^{13}C NMR data of carbonyl carbon at δ 179.2 indicated the carboxylic acid group in the structure of **5**. The ^{13}C/^{1}H NMR spectra of **6** were very similar to those of compound **1**, except that the C-6 methyl carboxylate group in **1** was replaced by a carboxylic acid group in **6**. This observation was confirmed by the absence of a OCH_3 signal in the ^{1}H and ^{13}C NMR spectra (δ 3.71 and 52.0) in **6**. Compound **6** also gave evidence for a methylenedioxyphenyl group from ^{1}H NMR signals appearing at δ 5.91 (2H, s, OCH_2O), 6.61 (1H, dd, J = 8.0, 1.6 Hz, H-11′), 6.67 (1H, d, J = 1.6 Hz, H-7′), and 6.72 (1H, d, J = 8.0 Hz, H-10′). Due to their optical inactivity, **4**−**6** are also considered to be racemic. The relative configurations of eight chiral centers at C-1, C-3, C-6, C-7, C-10, C-11, C-12, and C-13 in **4**, **5**, and **6** were determined by NOESY correlations and were similar to those of **1**−**3**. Thus, the structures of **4**−**6** were established as shown, and the compounds were named erythrophloins D−F, respectively.

Compound **7** was isolated as an optically inactive colorless oil. The HRESIMS gave a [M + Na]$^+$ ion peak at *m/z* 351.2663 (calcd 351.2664), consistent with a molecular formula of $C_{23}H_{36}O$. The ^{13}C NMR and DEPT spectra exhibited 23 signals, including two methyls, 10 methylenes, 10 methines, and a quaternary carbon. According to the 10 methines in the molecule, and without the characteristic four olefinic protons of **1**−**6** in the ^{1}H NMR spectrum of **7**, it was soon recognized that compound **7** does not have the same skeletal type as compounds **1**−**6**. The ^{1}H NMR data of **7** exhibited two *cis*-form olefinic protons appearing at δ 6.10 (1H, ddd, J = 8.0, 6.0, 1.0 Hz, H-10) and 6.20 (1H, ddd, J = 8.0, 6.4, 0.8 Hz, H-11). The structure of **7** was found to consist of 10 methines, including two olefinic carbons [C-10 (δ 130.3), C-11 (δ 132.3)] and a methylene [C-6 (δ 38.3)]. According to the ^{1}H−^{1}H COSY (Figure 1) and DEPT data, eight methines and one methylene were observed and linked together, forming a nine-carbon contiguous fragment (C-1, C-11, C-10, C-9, C-3, C-2, C-5, C-6, and C-7). As determined from the HMBC spectrum (Figure 1), long-range correlations between H-7/C-2 and H-1/C-5 revealed a five-membered ring and a six-membered ring composed of carbons C-1, C-2, C-5, C-6, C-7 and carbons C-1, C-11, C-10, C-9, C-3, C-2. The other six-membered ring was composed of carbons C-1, C-11, C-10, C-9, C-8, C-7, as ascertained by the ^3J correlations of H-8/C-1, C-6, and C-10 in the HMBC spectrum (Figure 1). Finally, ^3J correlations of H-6/C-4 and H-4/C-9 confirmed the presence of a four-membered ring. The absorption band at 1709 cm^{-1} in the IR spectrum exhibited a carbonyl group in the molecule. A methyl signal at δ 2.12 in the ^{1}H NMR spectrum and a carbonyl carbon at δ 209.2 in the ^{13}C NMR spectrum revealed the presence of a methyl

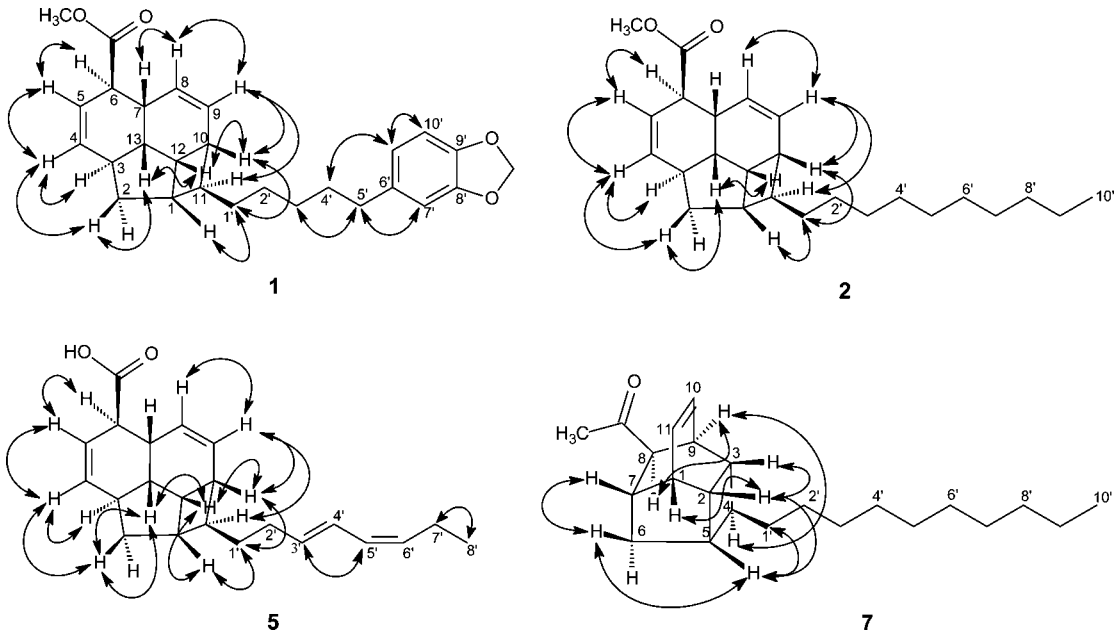

Figure 2. Key NOESY (H↔H) correlations of **1**, **2**, **5**, and **7**.

ketone group located at C-8, as confirmed through the ^3J correlations of H-7/C=O and CH_3/C-8. The signals of δ 0.88 (3H, t, J = 6.8 Hz, H-10′), 1.27 (16H, br s, H-2′-H-9′), and 1.52 (2H, m, H-1′) showed a decyl moiety located at C-4 by means of the HMBC ^3J correlations of H-5/C-1′ and H-4/C-2′ and the NOESY correlations between H-5/H-1′ and H-1′/H-3 (Figure 2). Because of its optical inactivity, **7** was considered to be a racemate.

The relative configuration of **7** was derived by a NOESY plot (Figure 2) in combination with biogenetic considerations[4] and comparison with endiandric acid C.[4] According to the NOESY spectrum, the α-orientation of H-9 could be confirmed by the key correlations of H-9/H-4, H-6α, and H-8. In contrast, other correlations between H-3/H-2, H-2/H-1 and H-5, H-5/H-6β, and H-6β/H-7 suggested the protons H-1, H-2, H-3, H-5, and H-7 are β-oriented. Thus, the relative configuration was assigned as *rel*-(1*R*,2*R*,3*R*,4*S*,5*S*,7*S*,8*R*,9*S*), and this compound was named beilcyclone A (**7**).

The antitubercular effects of the isolates from the roots of *B. erythrophloia* were tested in vitro against *M. tuberculosis* H37Rv. The clinically used antitubercular agent ethambutol (MIC = 6.25 μg/mL) was empolyed as the positive control. Among the endiandric acid analogues (**1**−**7**), only erythrophloin C (**3**) exhibited antitubercular activity (MIC = 50 μg/mL). Compound **3** has a phenyl group, with no other substitution in the side chain moiety. In addition, suberosol B (**8**) also showed antitubercular activity (MIC = 28.9 μg/mL) against *M. tuberculosis* H37Rv in vitro. This compound was reported earlier to inhibit the growth of P-388 murine lymphocytic leukemia cells.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanaco micromelting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1020 digital polarimeter. UV spectra were obtained on a JASCO UV-240 spectrophotometer in MeOH. IR spectra (KBr or neat) were taken on a Perkin-Elmer System 2000 FT-IR spectrometer. 1D (^{1}H, ^{13}C, DEPT) and 2D (COSY, NOESY, TOCSY, HSQC, HMBC) NMR spectra, using $CDCl_3$ as solvent, were recorded on a Varian Unity Plus 400 (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR) and Varian INOVA-500 (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR) spectrometer. Chemical shifts were referenced internally to the solvent signals in $CDCl_3$ (^{1}H, δ 7.26; ^{13}C, δ 77.0), with TMS as the internal standard. Low-resolution ESIMS were obtained on an API 3000 (Applied Biosystems); high-resolution ESIMS, on a Bruker Daltonics APEX II 30e spectrometer. Low-resolution EIMS were recorded on a Quattro GC/MS spectrometer having a direct inlet system. Silica gel (70−230, 230−400 mesh, Merck) was used for column chromatography, and silica gel 60 F-254 (Merck) was used for analytical and preparative TLC. A spherical C_{18} column (250 × 10 mm, 5 μm), a LDC-Analytical-III apparatus, and a UV−vis detector (SPD-10A, Shimadzu) were used for HPLC.

Plant Material. The roots of *B. erythrophloia* were collected from Mudan, Pingtung County, Taiwan, in February 2005, and a voucher specimen (Chen 1187) was deposited in the Herbarium of the Faculty of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan, Republic of China.

Extraction and Isolation. The dried roots (7.5 kg) were sliced and extracted with cold MeOH (30 L) three times. After concentration under reduced pressure, the MeOH extract was partitioned between EtOAc−H_2O (1:1) to obtain an EtOAc-soluble fraction (160 g), a H_2O-soluble fraction (70 g), and an insoluble fraction (23 g). A part of the active EtOAc fraction (100 g) was subjected to silica gel column chromatography (230−400 mesh, 2 kg), eluting with a gradient of *n*-hexane−EtOAc, to give 13 fractions (A1−A13). Fraction A4 (10.4 g) was applied to a silica gel column (230−400 mesh, 40 g), eluting with CH_2Cl_2−acetone (20:1), to give eight fractions (A4-1−A4-8). Fraction A4-3 (3.2 g) was chromatographed on a silica gel column (230−400 mesh, 100 g), eluting with *n*-hexane−acetone (10:1), to obtain suberosol B (**8**) (3.4 mg, R_f 0.48) and vanillin (5.0 mg, R_f 0.39). Fraction A4-6 (2.7 g) was chromatographed on a silica gel column (230−400 mesh, 50 g), eluting with CH_2Cl_2−acetone (10:1), to give eight fractions (A4-6-1−A4-6-8). Fraction A4-6-5 (28 mg) was further purified by preparative TLC (*n*-hexane−acetone, 10:1) to afford dioxamine (10.2 mg, R_f 0.65). Fraction A5 (2.66 g) was applied to a silica gel column (230−400 mesh, 40 g), eluting with a gradient of *n*-hexane−EtOAc, to obtain 11 fractions (A5-1−A5-11). Fraction A5-8 (41 mg) was applied to a RP-C_{18} column (10 g), eluting with acetone−H_2O (20:1), to afford **4** (4.3 mg, R_f 0.67). Fraction A5-10 (78 mg) was applied to a silica gel column (230−400 mesh, 10 g), eluted with *n*-hexane−acetone (20:1), to obtain five fractions (A5-10-1−A5-10-5). Fraction A5-10-4 (35 mg) was applied to a RP-C_{18} column (10 g), eluted with acetone−H_2O (10:1), to obtain five fractions (A5-10-4-1−A5-10-4-5). Fraction A5-10-4-3 (11.5 mg) was subjected to preparative HPLC (acetonitrile−H_2O, 20:1) to afford **5** (5.0 mg, t_R 19 min, 2 mL/min). Fraction A5-11 (52 mg) was applied to a RP-C_{18} column (10 g), eluted with MeOH−H_2O (20:1), to obtain six fractions (A5-11-1−A5-11-6). Fraction A5-11-4 (15 mg) was further purified by preparative TLC (acetone−H_2O, 10:1) to afford **3** (8.1 mg, R_f 0.48). Fraction A6 (1.01 g) was applied to a RP-C_{18} column (20 g), eluting with acetone−H_2O (3:1), to obtain four fractions (A6-1−A6-4). Fraction A6-2 (162 mg) was applied to a RP-C_{18} column (10 g), eluted with acetone−H_2O (8:1), to afford **1** (38.1 mg, R_f 0.34). Fraction A6-3 (22

mg) was applied to a silica gel column (230−400 mesh, 10 g), eluting with *n*-hexane−EtOAc (40:1), to afford **2** (3.6 mg, R_f 0.63). Fraction A8 (0.67 g) was applied to a RP-C_{18} column (10 g), eluting with acetone−H_2O (20:1), to give three fractions (A8-1−A8-3). Fraction A8-3 (60 mg) was subjected to preparative HPLC (acetonitrile−H_2O, 20:1) to afford methyl oleate (5.1 mg, t_R 25.4 min, 1 mL/min) and methyl palmitate (12.8 mg, t_R 26 min, 1 mL/min). Fraction A9 (10.3 g) was applied to a silica gel column (230−400 mesh, 250 g), eluting with a gradient of $CHCl_3$−EtOAc (10:1→5:1→2:1→1:1), EtOAc (100%), and MeOH (100%) to furnish 14 fractions (A9-1−A9-14). Fraction A9-2 (1.8 g) was applied to a RP-C_{18} column (25 g), eluting with acetone−H_2O (20:1), to give three fractions (A9-2-1−A9-2-3). Fraction A9-2-1 (29.2 mg) was further purified by preparative TLC (CH_2Cl_2−acetone, 20:1) to afford α-tocopheryl quinone (8.2 mg, R_f 0.49). Fraction A9-8 (40 mg) was further purified by preparative TLC (CH_2Cl_2−acetone, 10:1) to afford lupeol (5.5 mg, R_f 0.41). Fraction A9-10 (0.5 g) was applied to a RP-C_{18} column (10 g), eluting with acetonitrile−H_2O (20:1), to give four fractions (A9-10-1−A9-10-4). Fraction A9-10-2 (68 mg) was subjected to preparative HPLC (acetonitrile−H_2O, 20:1), to afford methyl linoleate (7.8 mg, t_R 22.7 min, 1 mL/min). Fraction A9-11 (1.7 g) was applied to a RP-C_{18} column (10 g), eluting with acetone−H_2O (10:1), to give eight fractions (A9-11-1−A9-11-8). Fraction A9-11-7 (103 mg) was applied to a RP-C_{18} column (10 g), eluting with acetone−H_2O (10:1), to give five fractions (A9-11-7-1−A9-11-7-5). Fraction A9-11-7-3 (27.5 mg) was further purified by preparative TLC (CH_2Cl_2−acetone, 5:1) to afford 3-*O*-acetyl-*epi*-betulinic acid (3.5 mg, R_f 0.67). Fraction A10 (20 g) was applied to a silica gel column (230−400 mesh, 400 g), eluting with a gradient of *n*-hexane−acetone (5:1→1:1), acetone (100%), and MeOH (100%), to obtain eight fractions (A10-1−A10-8) as well as pure **6** (6.7 mg, R_f 0.71). Fraction A10-3 (2.5 g) was applied to a silica gel column (230−400 mesh, 40 g), eluting with CH_2Cl_2−MeOH (15:1), to give nine fractions (A10-3-1−A10-3-9). Fraction A10-3-3 (78 mg) was applied to a RP-C_{18} column (10 g), eluting with acetone−H_2O (10:1), to obtain **7** (10.4 mg, R_f 0.66). Fraction A10-5 (56 mg) was applied to a silica gel column (2.8 g), eluting with CH_2Cl_2−acetone (5:1), to give 6β-hydroxystigmast-4-en-3-one (4.9 mg, R_f = 0.58) and 24(*S*)-3β-hydroxystigmast-5-en-7-one (4.5 mg, R_f = 0.61).

Erythrophloin A (1): yellowish oil; $[\alpha]^{25}_D$ ±0 (*c* 0.30, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 239 (3.21), 287 (3.71) nm; IR (neat) ν_{max} 1734 (ester C=O), 1039, 934 (OCH_2O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; ESIMS *m/z* 443 [M + Na]$^+$; HRESIMS *m/z* 443.2196 [M + Na]$^+$ (calcd for $C_{27}H_{32}O_4Na$, 443.2198).

Erythrophloin B (2): yellowish oil; $[\alpha]^{25}_D$ ±0 (*c* 0.30, $CHCl_3$); IR (neat) ν_{max} 1737 (ester C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 2; EIMS *m/z* 370 [M]$^+$; HREIMS *m/z* 370.2861 [M]$^+$ (calcd for $C_{25}H_{38}O_2Na$, 370.2872).

Erythrophloin C (3): yellowish oil; $[\alpha]^{25}_D$ ±0 (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 292 (3.84) nm; IR (neat) ν_{max} 1721 (ester C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; EIMS *m/z* 404 [M]$^+$; HRESIMS *m/z* 427.2610 [M + Na]$^+$ (calcd for $C_{28}H_{36}O_2Na$, 427.2612).

Erythrophloin D (4): yellowish oil; $[\alpha]^{25}_D$ ±0 (*c* 0.35, $CHCl_3$); IR (neat) ν_{max} 3468 (OH), 1721 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; EIMS *m/z* 354 [M]$^+$; HRESIMS *m/z* 377.2453 [M + Na]$^+$ (calcd for $C_{24}H_{34}O_2Na$, 377.2456).

Erythrophloin E (5): yellowish oil; $[\alpha]^{25}_D$ ±0 (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 256 (3.84), 267 (3.94), 278 (3.83) nm; IR (neat) ν_{max} 2600−3300 (COOH), 1701 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; ESIMS *m/z* 347 [M + Na]$^+$; HRESIMS *m/z* 347.1990 [M + Na]$^+$ (calcd for $C_{22}H_{28}O_2Na$, 347.1987).

Erythrophloin F (6): yellowish oil; $[\alpha]^{25}_D$ ±0 (*c* 0.45, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 230 (3.53), 285 (3.24) nm; IR (neat) ν_{max} 3437 (OH), 1697 (C=O), 1037, 923 (OCH_2O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 2; ESIMS *m/z* 429 [M + Na]$^+$; HRESIMS *m/z* 429.2046 [M + Na]$^+$ (calcd for $C_{26}H_{30}O_4Na$, 429.2042).

Beilcyclone A (7): colorless oil; $[\alpha]^{25}_D$ ±0 (*c* 0.41, $CHCl_3$); IR (neat) ν_{max} 1709 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.20 (1H, ddd, *J* = 8.0, 6.4, 0.8 Hz, H-11), 6.10 (1H, ddd, *J* = 8.0, 6.0, 1.0 Hz, H-10), 3.02 (1H, dt, *J* = 6.0, 4.0 Hz, H-9), 2.78 (1H, d, *J* = 4.0 Hz, H-8), 2.68 (1H, m, H-7), 2.66 (1H, m, H-1), 2.35 (1H, dt, *J* = 7.2, 6.0 Hz, H-2), 2.23 (1H, br t, *J* = 6.0 Hz, H-5), 2.12 (3H, s, $COCH_3$), 1.89 (1H, ddd, *J* = 12.8, 7.7, 5.0 Hz, H-6β), 1.64−1.70 (2H, m, H-3, 4), 1.52 (2H, m, H-1′), 1.46 (1H, d, *J* = 12.8 Hz, H-6α), 1.27 (16H, br s, H-2′-H-9′), 0.88 (3H, t, *J* = 6.8 Hz, H-10′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 209.2 (C=O), 132.3 (C-11), 130.3 (C-10), 57.8 (C-8), 42.2 (C-1), 40.3 (C-5), 40.2 (C-2), 39.9 (C-3), 39.6 (C-4), 38.3 (C-6), 36.9 (C-7), 36.3 (C-1′), 35.5 (C-9), 31.9 (C-8′), 29.4−29.8 (C-3′−C-7′), 28.3 ($COCH_3$), 27.3 (C-2′), 22.7 (C-9′), 14.1 (C-10′); ESIMS *m/z* 351 [M + Na]$^+$; HRESIMS *m/z* 351.2663 [M + Na]$^+$ (calcd for $C_{23}H_{36}ONa$, 351.2664).

Antitubercular Activity Assay. Antitubercular assays were carried out in accordance with methods discussed in the literatures.[21,22]

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